Development of azaquinazoline anti-*Wolbachia* drugs for veterinary zoonotic filariasis

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Dirofilaria immitis is a mosquito-borne filarial nematode causing potentially lethal heartworm disease in cats and dogs. *Dirofilaria spp.* also cause zoonotic infections and pathologies in humans. Currently, control of veterinary dirofilariasis relies on chemoprophylaxis using macrocyclic lactone drugs, however, resistant isolates are increasing and resulting in preventative treatment failure.

Wolbachia is an essential endosymbiont necessary for development, reproduction, and survival of filarial nematodes. Targeting *Wolbachia* within filarial parasites using second generation tetracyclines is a relatively new curative treatment approach for both human and veterinary filariasis. Doxycycline, whilst effective at sterilising worms and leading to the gradual death of adult filariae, requires long treatment durations, can cause dysbiosis side-effects and raises antibiotic stewardship concerns, precluding the widespread use of tetracyclines in companion animals. We have developed a new azaquinazoline class of anti-*Wolbachia* drug with no broad-spectrum antibiotic activities. The first-in-class clinical candidate, AWZ1066S, mediates 5-day curative activity in human filariasis infection models. Here we report that AWZ1066S also mediates complete chemoprophylaxis (blocking adult infections) in a *Brugia malayi* mouse model when administered at day 1 and 29 after larval infection. We therefore selected four back-up azaquinazoline analogues of AWZ1066S for comparative *in vivo* efficacy against *D. immitis,* based on drug-like properties and *in vitro* anti-*Wolbachia* activity. We determined three analogues had equipotent or enhanced *Wolbachia* depletions in comparison to AWZ1006S using one-day oral exposures. One candidate, AWZ1023, was selected for further development in a proof-of-concept dog chemoprophylactic pilot study. Three dogs receiving twice-daily intramuscular dosing of AWZ1023 (day 1 and 29) had complete absence of adult heartworm at study termination (day 177), demonstrating the effectiveness of AWZ1023 at blocking juvenile nematode development.

In conclusion, this study provides an early proof-of-concept that azaquinazolines targeting nematode *Wolbachia* are a promising new class of drug for the treatment and prevention of veterinary zoonotic filarial infections, providing a potential alternative to the current reliance on macrocyclic lactone drugs.